NiPERA INC.
Nickel Producers Environmental Research Association

October 19, 1998

Dr. C. W. Jameson
National Toxicology Program
Report on Carcinogens
MD EC-14
P.O. Box 12233
Research Triangle Park, NC 27709

Dear Dr. Jameson:

The attached document is an integrated discussion of the carcinogenic potential of metallic nickel and the major classes of nickel compounds. This information is a supplement to the data already provided to the NTP by NiPERA for consideration in deliberating the proposal to list "Nickel and Nickel Compounds" as "Known Human Carcinogens" in the NTP's Ninth Biennial Report on Carcinogens. In our view, the fundamental problem with the NTP proposal is that it fails to recognize the critical importance of metal speciation in evaluating the toxicity and potential carcinogenicity of the various forms of nickel.

Each compound or species of nickel has its own physico-chemical properties that dictate how it behaves under a given set of conditions, including interactions with biological organisms. Thus, the fact that one form of nickel may be carcinogenic *via* a particular route of exposure (*e.g.*, inhalation) does not mean that a second nickel species will be carcinogenic as well or that the first nickel species will be carcinogenic *via* a different route of exposure (*e.g.*, ingestion). This observation holds true even if the free metal ion is assumed to be the active carcinogenic agent. Nickel compounds have different biological effects since the different physico-chemical properties of various forms of nickel will largely determine the extent to which the free metal ion (Ni^{2+}) can be made bioavailable to the relevant biological site within a cell for cancer to be initiated (*i.e.*, the cell nucleus) .

Consideration of the unique physico-chemical properties of different nickel compounds and the resultant unique toxicologic profiles of these compounds has been made by regulatory and safety organizations. For example, the ACGIH has recently finalized nickel species specific Threshold Limit Values (TLVs) and carcinogen classifications for metallic nickel, nickel carbonyl, and soluble, insoluble, and sulfidic nickel compounds. The European Commission is currently evaluating a similar grouping of nickel and nickel compounds for setting European occupational exposure levels. In North America, the U.S. EPA Office of Drinking Water, Health Canada, and industry have jointly commissioned a risk assessment of the oral and inhalation carcinogenic potential of soluble nickel compounds as a distinct nickel compound class.

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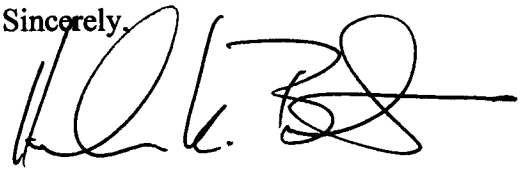
Against this background and as detailed in the attached document, the proposal to sweep metallic nickel and all nickel compounds into the single category of Known Human Carcinogens, is inconsistent with the chemical, epidemiological, and toxicological literature. In addition, it is at odds with the best current understanding of the likely mechanism of nickel-related carcinogenicity. Consequently, NiPERA proposes the following classifications for metallic nickel and the major nickel compounds or classes:

- Sulfidic nickel (including nickel subsulfide) – "known human carcinogen,"
- A distinction should be drawn between two groups of nickel oxides:
  - Mixtures of Ni-Cu oxides, high-temperature NiO, and low-temperature NiO found in nickel refineries – "known human carcinogen,"
  - Silicate oxides and complex nickel oxides (devoid of copper) found in nickel using industries – "reasonably anticipated to be a carcinogen,"
- Soluble nickel compounds (such as hydrated nickel sulfate and nickel chloride) – no NTP classification is justified,
- Metallic nickel, no NTP classification is justified, and
- Nickel carbonyl no NTP classification is justified.

A detailed discussion of these recommendations appears in the attached document. NiPERA is always available to provide any further documentation that the NTP may need in its deliberations. Thank you for this opportunity for involvement in the NTP process.

Sincerely,



Hudson K. Bates, Ph.D., DABT
Senior Health Scientist

Enclosure

cc.

Comments of the Nickel Producers Environmental Research Association on the National Toxicology Program Carcinogen Classification of Nickel and Nickel Compounds

October 13, 1998

1. Executive Summary

The U.S. National Toxicology Program (NTP) is reviewing the database on the potential carcinogenicity of nickel and nickel compounds. In the NTP's Eighth Report on Carcinogens, *Nickel and Certain Nickel Compounds* (*i.e.*, not including water soluble nickel compounds) were listed as substances that are "*reasonably anticipated to be a carcinogen*". The new proposal for the Ninth Report on Carcinogens would list *Nickel and Nickel Compounds* as substances that are "*known human carcinogens.*" NiPERA believes that this change would be scientifically unjustified and inappropriate.

NiPERA's major objection to the NTP's proposal to list *Nickel and Nickel Compounds* as "*known human carcinogens*" in the Ninth Biennial Report on Carcinogens is that it fails to recognize the **critical importance of speciation in evaluating the toxicity and potential carcinogenicity of the various forms of nickel**. Each compound or species of a metal, like nickel, has its own physico-chemical properties that dictate how it behaves under a given set of conditions, including interactions with biological organisms. Thus, the fact that one form of nickel may be carcinogenic via a particular route of exposure (*e.g.*, nickel subsulfide by inhalation) does not mean that a second nickel species will be carcinogenic as well or that the first nickel species will be carcinogenic via a different route of exposure (*e.g.*, ingestion). For nickel and its compounds, this observation holds true even if the free metal ion is assumed to be the active carcinogenic agent, because the different physico-chemical properties of various forms of the metal will largely determine the extent to which the free metal ion can be made bioavailable and delivered to a relevant biological site (*e.g.*, the nucleus of a lung epithelial cell).

Examination of the *in vitro*, animal, and epidemiologic data pertaining to commercially relevant nickel compounds[1] confirms that these compounds have very different biological behaviors, particularly with regard to respiratory carcinogenicity. Nickel subsulfide is likely to be carcinogenic to humans. Soluble nickel compounds, by themselves, have not been demonstrated to be carcinogenic to humans, although an enhancing (promoter) effect on other carcinogens is possible. High concentrations of oxidic nickel mixtures (*i.e.*, Ni-Cu oxides mixed with low-temperature [black] and high-temperature [green] NiO) appear to be carcinogenic in epidemiologic studies of nickel refinery workers. Exposures to nickel silicates-oxides and complex nickel oxides devoid of copper have not resulted in excess cancer risks in other human cohorts. Exposure to metallic nickel particles in the workplace does not appear to pose a respiratory carcinogenic risk for humans. Finally, nickel carbonyl is so acutely toxic that it is used in closed systems and humans are typically exposed only in accident scenarios. The high acute toxicity of nickel carbonyl has limited its examination for carcinogenic effects. The human and animal data on the potential carcinogenicity of nickel carbonyl are scant and only non-standard animals studies with exposures above the Maximum Tolerated Dose (MTD) have yielded evidence of a carcinogenic effect.

Against this background, NiPERA believes that the NTP proposal to sweep metallic nickel and all nickel compounds into the single category of "*known human carcinogens*" is inconsistent with both the epidemiological and toxicological data and is at odds with the best current understanding of the likely mechanism of nickel-related carcinogenicity.

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[1] The classes of nickel compounds discussed in this paper are: metallic nickel, oxidic nickel (including nickel oxides, hydroxides, silicates, carbonates, and complex nickel oxides), sulfidic nickel (including nickel sulfide and subsulfide), water soluble nickel compounds (including hydrated forms of nickel acetate, sulfate, chloride, *etc.*), and nickel carbonyl. Metallic, oxidic, and sulfidic nickel compounds and nickel carbonyl are insoluble in water.

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2. Introduction

The U.S. National Toxicology Program (NTP) is reviewing the database on the potential carcinogenicity of nickel and nickel compounds. In the NTP's Eighth Report on Carcinogens, *Nickel and Certain Nickel Compounds* (*i.e.*, not including water soluble nickel compounds) were listed as substances that are "*reasonably anticipated to be a carcinogen*". The new proposal for the Ninth Report on Carcinogens would list *Nickel and Nickel Compounds* as substances that are "*known human carcinogens.*" NiPERA believes that this change would be scientifically unjustified and inappropriate.

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Historically, inhalation exposure to very high concentrations of certain nickel compounds in the nickel producing industry has been associated with an excess of respiratory cancer. It should be noted that only respiratory tumors have been consistently associated with these exposures and solely by the inhalation route of exposure. To understand the risks associated with exposures to nickel compounds, consideration should be given to the respiratory carcinogenic potential of the individual nickel species and the influence of particle size and mixed exposures. Examination of the *in vitro*, animal, and epidemiologic data pertaining to the four commercially relevant classes of nickel compounds[2] confirms that these compounds have very different biological behaviors, particularly with regard to respiratory carcinogenicity. Nickel subsulfide is likely to be carcinogenic to humans. Soluble nickel compounds, by themselves, have not been demonstrated to be carcinogenic to humans, although an enhancing (promoter) effect on other carcinogens is possible. High concentrations of oxidic nickel mixtures (*i.e.*, Ni-Cu oxides mixed with low-temperature [black] and high-temperature [green] NiO) appear to be carcinogenic in epidemiologic studies of nickel refinery workers. Exposures to nickel silicates-oxides and complex nickel oxides devoid of copper have not resulted in excess cancer risks in other human cohorts. Exposure to metallic nickel particles in the workplace does not appear to pose a respiratory carcinogenic risk for humans. Finally, nickel carbonyl is so acutely toxic that it is used in closed systems and humans are typically exposed only in accident scenarios. The high acute toxicity of nickel carbonyl has limited its examination for carcinogenic effects. The human and animal data on the potential carcinogenicity of nickel carbonyl are scant and only non-standard animals studies with exposures above the Maximum Tolerated Dose (MTD) have yielded evidence of a carcinogenic effect.

A brief review of the epidemiologic, animal and *in vitro* data pertinent to the understanding of the inhalation carcinogenicity of nickel and its compounds is presented in this report. Based on these data, a possible mechanistic model for the carcinogenicity of nickel compounds is discussed [(1)]. The carcinogenic potentials of sulfidic nickel (*e.g.*, nickel subsulfide), oxidic nickel compounds (with particular emphasis on high temperature [green] nickel oxide), soluble nickel compounds (*e.g.*, nickel sulfate hexahydrate),

[2] The four classes of nickel compounds discussed in this paper are: metallic nickel, oxidic nickel (including nickel oxides, hydroxides, silicates, carbonates, and complex nickel oxides), sulfidic nickel (including nickel sulfide and subsulfide) and water soluble nickel compounds (including hydrated forms of nickel acetate, sulfate, chloride, etc.). Metallic, oxidic and sulfidic nickel compounds are insoluble in water.

metallic nickel, and to a lesser extent nickel carbonyl, are considered within the framework provided by this model.

Against this background, NiPERA believes that the NTP proposal to sweep metallic nickel and all nickel compounds into the single category of "*known human carcinogens*" is inconsistent with both the epidemiological and toxicological data and is at odds with the best current understanding of the likely mechanism of nickel-related carcinogenicity.

3. EXPERIMENTAL DATA

3.1. EPIDEMIOLOGIC DATA

Epidemiologic data from nickel workers are difficult to interpret because of mixed exposures to not only different nickel compounds but also to other inorganic compounds (arsenic, cobalt, strong acid mists) and to organic combustion products [2]. In addition, exposure measurements are sparse, very little chemical speciation and particle size information is available, and the confounding effects of cigarette smoking on respiratory cancers have not been adequately studied. Nevertheless, with the continued acquisition of new epidemiologic data, a clearer picture is emerging with respect to the likely role that different nickel species play in human respiratory carcinogenesis. In later sections, it will be noted that this picture is largely in agreement with what is known about these compounds from animal and *in vitro* studies.

Studies of past exposures and cancer mortality reveal that only respiratory tumors have been consistently associated with inhalation exposure to certain nickel compounds. Data from ten different cohorts were presented in the report of the International Committee on Nickel Carcinogenesis in Man (ICNCM) [2]. These cohorts included approximately 80,000 workers involved in nickel operations (mostly mining, smelting, and refining, but some nickel alloy production and miscellaneous applications as well) located in the United States, Canada, England, Wales, Norway, Finland and New Caledonia.

Of the examined workers, less than 10% had clear excess respiratory cancer risks. The excess risks were confined to workers in certain types of refining operations. Only slightly elevated risks of respiratory cancer were seen in some (but not all) smelting and mining workers; these appeared to be attributable to other causes [3,4]. There was no evidence of risk for workers in the manufacturing of barrier material for gaseous diffusion (uranium enrichment process), nor in workers involved in alloy production. An additional 50,000 workers in nickel-using industries and applications (stainless steel and nickel alloy production, welding, and plating) have given no evidence of excess respiratory cancer risks from exposures to metallic and/or complex nickel oxides largely free of copper [5-9].

Thus, of the large number of nickel-exposed workers comprising a variety of occupations, only a small proportion have shown excess respiratory cancer risks. Nickel-related cancer risks appear to have been confined to certain types of refining operations, most of which are no longer in existence today. No nickel-related excess respiratory cancer risks have been found in any nickel-using industry workers.

3.1.1. Sulfidic Nickel

The ICNCM report [2] concluded that much of the excess respiratory cancer risk in workers involved in certain types of nickel refining operations appeared to be associated with exposure to a mixture of sulfidic and oxidic nickel compounds at high concentrations ($\geq$ 10 mg Ni/m^3). In the case of sulfidic nickel, both lung and nasal cancers were associated with exposure to this nickel compound in Canadian sinter plant workers. In refinery workers in Clydach, Wales excess lung cancers were associated with high cumulative exposures to sulfidic nickel and low-level exposures to other nickel compounds. It should be noted that the risks of developing respiratory cancers in this cohort dramatically dropped after 1930 despite the continued presence of some high levels of sulfidic nickel into the late 1930s, suggesting that other factors (*e.g.,* possible presence of arsenic) could have contributed to the cancer risks seen in these workers. However, clear evidence of respiratory carcinogenicity in animals administered nickel subsulfide (see

below) indicates that the association of exposures to sulfidic nickel and lung and nasal cancer in humans is likely to be causal.

3.1.2. Oxidic Nickel

With respect to oxidic nickel, excess lung and nasal cancers reported in refinery workers in Clydach and in Kristiansand, Norway who were exposed to high concentrations of oxidic nickel (mainly as nickel copper oxides, but with the possible presence of both high-temperature and low-temperature NiO as well), strongly suggests that these forms of oxidic nickel are likely human respiratory carcinogens[3]. Conversely, in nickel-using industry workers exposed to metallic nickel and/or complex nickel oxides free of copper, with no exposure to sulfidic nickel, there have been no nickel-related excess risks of respiratory cancer. Likewise, nickel production workers involved in the mining and smelting of lateritic ores have shown no nickel-related excess respiratory cancer risks. The oxidic nickel to which these workers were exposed would have mainly been nickel silicates-oxides and complex nickel oxides devoid of copper. It should be mentioned that oxidic nickel exposures in the latter groups were considerably lower than those experienced by workers in certain types of nickel refining operations. It is uncertain, therefore, whether the lack of increased respiratory cancer risk in these workers was due to the low concentrations of oxidic nickel to which they were exposed and/or to the physicochemical properties (including particle size) of the particular oxidic nickel compounds present.

3.1.3. Soluble Nickel

The role of soluble nickel in respiratory carcinogenesis is less evident than that of sulfidic and certain oxidic nickel compounds. Comparisons of electrolysis workers at Port Colborne, Canada and Kristiansand, Norway reveal that only Kristiansand workers had excess lung cancers. Because of differences in processes, the Kristiansand workers were thought to be exposed to slightly higher levels of soluble nickel and also to handle approximately seven times more insoluble nickel (per unit of soluble nickel) than those at Port Colborne. In addition, basic nickel carbonate (water insoluble) was included in the soluble compounds category at Kristiansand, whereas it was classified as insoluble at Port Colborne. While the amounts involved were not large, they would have exaggerated the differences in exposure to soluble compounds between the two operations. In another cohort of hydro-metallurgical workers at Clydach that had high cumulative exposure to soluble forms of nickel but low exposures to oxidic and sulfidic forms of nickel, there was no evidence of increased risks of respiratory cancer. From these studies, the ICNCM Report concluded that, while there was evidence that soluble nickel exposure ($\geq$ 1 mg Ni/m^3) could increase the risk of respiratory cancers, the effect might be one of enhancing risks associated with co-exposure to less soluble forms of nickel or other non-nickel compounds.

Recent studies have provided supportive evidence for the possible role of soluble nickel as a promoter of carcinogenicity. In particular, in a recent study of the Kristiansand cohort that has updated cancer morbidity, newly available information on the smoking characteristics of the workers has been included [(10)]. A synergistic lung cancer response between smoking and exposure to a mixture of soluble and insoluble nickel compounds was observed. In the small number of nickel-exposed workers who did not smoke, there was no evidence that nickel exposure increased the risk for lung cancer. A similar lack of excess respiratory cancers was noted in a 1996 cancer mortality study in a relatively small population of nickel platers exposed solely to nickel chloride and sulfate mists [(9)]. The results from these two studies are consistent with those of the ICNCM Report.

In a 1998 study of Finnish refinery workers exposed predominantly to soluble nickel three nasal cancer cases were identified and a 2-fold increase in lung cancer risk was found in nickel workers with more than

[3] It should be noted that these workers were also exposed to various levels of metallic, sulfidic and/or soluble nickel compounds, since no workplace in the producing industry had "pure" exposure to any individual nickel compound.

20 years employment [11]. Unfortunately, smoking data are unavailable for these workers. As indicated in the above study on Norwegian electrolysis workers, such data would be helpful in interpreting the significance of the lung cancers seen in these workers. In the case of the observed nasal cancers, even though the Finnish workers were predominantly exposed to soluble nickel during their employment at the refinery, their previous job experiences, as well as concomitant exposures to insoluble nickel compounds and acid mists, make the establishment of a causal association with soluble nickel compounds difficult.

Taken together, the epidemiologic results from all the above studies are most consistent with soluble nickel compounds enhancing, rather than initiating, cancer. The animal data on soluble nickel compounds strongly support this interpretation (see next section).

3.1.4. Metallic Nickel

The ICNCM Report found no evidence that exposure to metallic nickel in industrial plants increased respiratory cancer risk. The lack of excess respiratory cancer risks in workers at a gaseous diffusion barrier manufacturing plant was particularly notable as these workers were exposed solely to metallic nickel. Likewise, in a recent update of a study on 715 hydrometallurgical workers in Canada, no excess lung or nasal cancers was reported [12]. Although the size of the cohort was small, exposures in this plant were solely to nickel concentrates and metallic nickel. In a recent study of nickel alloy workers, Redmond and coworkers updated the cancer mortality data from more than 30,000 people employed in 13 nickel alloy plants in the U.S.A. Exposures were primarily to metallic nickel and complex nickel oxides devoid of copper. No excess mortality rates were observed for respiratory cancers in these workers when compared to local population rates [5,6]. Examination of the available data shows that, even in the past, exposures to metallic nickel have generally been low ($\leq$ 1 mg Ni/m^3) compared to exposures to other nickel compounds found in certain types of nickel refining operations. The overwhelming lack of epidemiologic carcinogenic evidence for metallic nickel could be due to the combination of low-dose exposures, the particle size of the metallic nickel found in the workplace, and the limited bioavailability of the nickel ion from nickel metal itself. It is clear then, that under past and current industrial practices, exposure to metallic nickel does not pose a respiratory carcinogenic risk for humans.

3.1.5. Nickel Carbonyl

The severe acute toxicity effects of nickel carbonyl have been recognized for decades. It is because of this acute toxicity that short-term exposure limits are usually set. The only human study investigating the possible health effects of nickel carbonyl involved the examination of causes of death in 69 men who worked at Clydach, Wales from 1933 to 1966[13]. Their SMR for lung cancer was 152 and was not considered to be statistically significant. The presence of other confounding exposures at Clydach was not considered in this study.

3.2. ANIMAL DATA

Animal data are often useful in helping to elucidate mechanisms of carcinogenesis. As noted above, this is particularly true in the case of nickel and its compounds where the animal data are in good agreement with the human lung carcinogenicity data. The ICNCM Report, recognizing the limitations of human studies involving mixed exposures, pointed out the importance of the results of animal carcinogenesis studies (using inhalation as the route of exposure) to help understand the human health risks associated with individual nickel compounds. It should be noted that under the conditions used in the studies, none of the rodent species showed evidence of nasal tumors after inhalation exposure to any one of the nickel compounds tested. The animal data are reviewed herein.

3.2.1. Nickel Subsulfide

In a 1974 study, inhalation of nickel subsulfide (Ni_3S_2) resulted in the induction of lung tumors in rats [14]. The U.S. National Toxicology Program (NTP) recently completed two-year inhalation cancer bioassays in rats and mice with three nickel compounds, including nickel subsulfide [15,16]. In the nickel subsulfide study, rats were exposed to 0, 0.1 or 0.7 mg Ni/m^3; mice were exposed to 0, 0.4, or 0.8 mg Ni/m^3. After two years exposure, there was clear evidence of carcinogenic activity in male and female rats, with a dose-dependent increase in lung tumor response. No evidence of carcinogenic activity was detected in male or female mice. No nasal tumors were detected in rats or mice, but various non-malignant lung effects were seen.

3.2.2. Oxidic Nickel

In the case of oxidic nickel, few properly designed chronic inhalation studies had been performed prior to the NTP studies [15,17]. The first inhalation studies that were carried out on hamsters and rats with different nickel oxides were either negative or inconclusive due to high mortality at toxic concentrations [18-23].

In the recently completed NTP study [15,17], rats were exposed to high temperature, green NiO (calcined at 1,350 °C) at concentrations of 0, 0.5, 1.0, or 2.0 mg Ni/m^3. After two years, no increased incidence of tumors was observed at the lowest exposure level in rats. At the intermediate and high concentrations, 12 out of 106 rats and 9 out of 106 rats, respectively, presented with either adenomas or carcinomas. These numbers were not statistically different from those seen both in the control and low dose groups, but were statistically significant compared to historical controls (cancer incidence in ~200 control rats per sex used in previous NTP studies). Therefore, the NTP concluded that there was some evidence of carcinogenic activity in rats. NTP also found equivocal evidence of carcinogenicity in female mice based on excess tumors found in animals exposed to 1 but not 2 or 4 mg Ni/m^3. Other findings in rodents included inflammation and pigmentation in the lung and lymphoid hyperplasia and pigmentation in the bronchial lymph nodes. No nasal tumors were observed in rats or mice.

Two clearance studies of high temperature, green NiO particles from the respiratory tract of rats and mice showed impaired clearance of NiO after two to six months exposure to the same concentrations used in the NTP studies [24-26]. These results indicate that impairment of lung clearance was likely present in the NTP rats at the concentrations at which tumors were found.

It has been shown in rats that prolonged exposures to high concentrations of particles of low toxicity can result in lung tumors independent of the composition of the particles [27,28]. The mechanism for tumor induction involves an impairment of lung clearance that leads to chronic inflammation. Chronic inflammation can result in enhanced cell proliferation, and, indirectly, in increases in mutations through the action of oxygen radicals produced by the activated inflammatory cells [29-32]. The tumors found in rats exposed to high concentrations of high temperature green nickel oxide in the NTP studies could have been the result of the indirect particle effect described above, rather than resulting from the direct genotoxic effects of Ni^{2+}. It is uncertain at present, whether the induction of tumors secondary to a particle effect observed in rats could occur in humans, since no excess tumor incidence has been observed in workers exposed to very high concentrations of low solubility and low toxicity dusts [33].

In evaluating the carcinogenicity of oxidic nickel compounds, it is important to consider the concentration and physicochemical characteristics of the particles (including particle size). The physicochemical characteristics of oxidic nickel produced by different processes as well as the presence of other metals (*e.g.,* Ni-Cu oxides) may result in different biological activities. For example, a nickel oxide produced at lower calcining temperatures than the green NiO may have increased solubility resulting, perhaps, in enhanced toxicity as well as clearance. Depending on the balance of these effects, the ultimate result may be an increase or decrease in the respiratory carcinogenic potential of the various oxidic nickel compounds relative to high temperature, green nickel oxide.

3.2.3. Soluble Nickel

No inhalation studies with soluble nickel compounds had been conducted prior to the NTP studies. Soluble nickel compounds gave consistent negative results by oral [34-37] and intramuscular [38-41] routes of exposure; only intraperitoneal injection studies gave positive results with nickel acetate [42-45]. Injection studies are not appropriate to evaluate hazards for predicting human risk from inhalation exposures. This is due to the fact that injection bypasses natural protective mechanisms and causes unrealistically high spikes of exposure to occur in various organ systems.

In the recently conducted NTP inhalation study [16,46], rats were exposed to $NiSO_4 \cdot 6H_2O$ at concentrations up to 0.11 mg Ni/m^3; mice were exposed to up to 0.22 mg Ni/m^3. These concentrations were chosen based on the toxicity observed in the 13-week studies and corresponded to the maximum tolerated doses (MTD). After two years of continuous exposure, there was no evidence of lung or nasal carcinogenic activity in mice or rats. Various combinations of non-carcinogenic lung effects were seen in both sexes in rats and mice. Overall, the non-carcinogenic effects were similar to those seen with the other two nickel compounds.

3.2.4. Metallic Nickel

A limited number of animal inhalation studies with elemental nickel powder have not indicated carcinogenicity in rats or hamsters [47]. In one intratracheal instillation study, 9 mg Ni/rat of nickel powder (unspecified particle size) produced malignant lung tumors in rats [48]. However, the relevance of such a route of administration for humans is highly questionable, given that the lung burden by intratracheal instillation is massive, potentially overloading lung clearance mechanisms and affecting the animal's ability to eliminate the material. Intratracheal instillation of 10 mg nickel powder did not induce tumors in hamsters [49].

3.2.5. Nickel Carbonyl

Published studies on the carcinogenicity of nickel carbonyl were all performed prior to present day standardized testing protocols and because of the extreme acute toxicity of this material, more recent studies have not been conducted.

Sunderman and co-workers[50] exposed 64 male rats to 30 mg/m^3 and 32 male rats to 60 mg/m^3 of nickel carbonyl vapor three times a week for one year. Only 20 test animals survived the study. In a second study 80 rats were exposed to a single dose of 250 mg/m^3 and observed for effects for two years. Of the 176 animals exposed to nickel carbonyl in these two experiments, only 9 survived for two years, and of these, 4 had tumors.

In later studies, Sunderman and Donnelly[51] exposed 285 male rats to 600 mg/m^3 of nickel carbonyl for 30 minutes. Only 71 rats survived for longer than 3 weeks, with roughly equivalent numbers of tumors found in both the exposed and the control animals. The experiments of Sunderman and coworkers are the only animal studies linking nickel carbonyl to respiratory cancer. Thus the high rate of early mortality, the fact that in some studies the controls also developed tumors, as well as the possible secondary effects of acute nickel carbonyl poisoning preclude definitive evaluation of carcinogenicity.

3.3. IN VITRO STUDIES

In vitro, the Ni^{2+} ion does not behave like a typical mutagen; it does not show high affinity for DNA and lacks mutagenicity in most bacterial and mammalian assays [52-59]. Only chromosomal aberration assays (indicative of chromosomal damage) have been positive with nickel compounds *in vitro* [60-67] and *in vivo* [68-73].

However, *in vitro* cell transformation assays have been positive with soluble and insoluble nickel compounds [54,55,74-77]. It was shown that endocytosis by target cells was likely to play an important role in the *in vivo* transforming potential of nickel compounds [77]. Endocytized particles release Ni^{2+} ions and are transported to the nuclear membrane where the endocytic vesicles deliver Ni^{2+} ions in close proximity to the chromosomes [78]. Some of the characteristics of insoluble nickel compounds that increase their ability to be endocytized include: crystalline nature, negative surface charge, 2-4 µm range particle size, and low solubility in biological fluids [79-81]. Even though water soluble nickel compounds are not endocytized, they are positive in *in vitro* transformation assays due to the persistent high concentration of Ni^{2+} ions that can be achieved in the cell culture medium. The high nickel gradient in the medium allows Ni^{2+} ions to concentrate at the nuclear target sites. However, *in vivo*, Ni^{2+} ions from soluble compounds are unlikely to be bioavailable due to their rapid clearance from the lung and excretion in urine ($t_{1/2}$ in rats of ~ 2-3 days[24]) .

The Ni^{2+} ion present at nuclear sites has been shown *in vitro* to bind to proteins within heterochromatin regions of DNA [82-84]. This binding may enhance DNA condensation and methylation in nearby regions [85-87] and may result in nickel-mediated induction of oxidative DNA damage [44,82, 88-92]. These actions could have similar effects on senescence or tumor suppressor genes; the former, by diminishing gene expression, the latter by resulting in deletion of these genes [86,87,93-96].

4. Mechanistic Model Related to the Carcinogenicity of Nickel Compounds

There are two components that may contribute to the development of lung tumors by certain nickel compounds [1]: (1) heritable changes in gene expression and (2) cell proliferation. Heritable changes in gene expression can be the result of: (i) genetic changes such as mutations (changes in DNA sequence) or chromosomal aberrations (changes at the chromosome level), and (ii) epigenetic changes that affect gene expression without altering DNA sequences. Nickel compounds have not been shown to directly induce mutations, but some nickel compounds are able to cause heritable chromosomal aberrations and epigenetic changes through methylation. These direct effects could be specific for Ni^{2+} ions and dependent on its bioavailability (the delivery of the nickel ion to sites within the nuclei of the target cells). In addition, some nickel compounds may have indirect effects as a consequence of an inflammatory response. The indirect effects could be attributed to DNA damage caused by oxygen radicals and are not specific to nickel.

Cell proliferation is required to convert DNA lesions into mutations and is also involved in clonal expansion of the initiated cell population, a factor that increases the probability of occurrence of a second mutating event. Only sustained increases in cell proliferation, as seen in chronic exposures, are likely to be significant in carcinogenesis [97]. Expression of pro-inflammatory cytokines was found to be increased in lungs of rats and mice after subchronic exposure to nickel subsulfide [26]. Cell proliferative responses in alveolar epithelial cells from rats and mice exposed to high temperature green NiO, paralleled the inflammatory responses [26]. Collectively, these studies suggest that some nickel compounds can stimulate cell proliferation *in vivo*. Again, this effect may not be specific for nickel compounds, and it could be similar for other substances that can induce proliferative responses. Both components: heritable changes in gene expression and cell proliferation, are needed for tumor development.

5. Carcinogenic Assessment of Individual Nickel Compounds

5.1. SULFIDIC NICKEL

The human data provide evidence of an association of excess respiratory cancer risk with inhalation of aerosols containing high concentrations of sulfidic nickel (>10 mg Ni/m^3). Positive animal carcinogenicity results from inhalation exposure to nickel subsulfide have been found in rats (with evidence of dose-response).

Nickel subsulfide particles need to be oxidized to release Ni^{2+} ions. Nickel subsulfide is quite insoluble in water, but shows enhanced release of Ni^{2+} ions in biological fluids. *In vivo*, since nickel subsulfide is likely to be readily endocytized by the target cells, this compound is likely to affect both components of the carcinogenic process (induction of heritable changes and increases in cell proliferation). Because of its enhanced "solubility" in biological fluids, efficient delivery of Ni^{2+} ions to the target site within the cell nucleus is likely. The release of Ni^{2+} ions on the alveolar surface can result in cell toxicity and directly induce inflammation and proliferation of initiated cells.

With regard to carcinogenicity assessment, an NTP category of "*known human carcinogen*" seems appropriate. Because nickel subsulfide may efficiently affect both components of the carcinogenic process, this compound appears to present the highest respiratory carcinogenic potential relative to other nickel compounds.

5.2. OXIDIC NICKEL

Historical human data indicate that inhalation exposure to high concentrations (>10 mg Ni/m^3) of oxidic nickel (consisting of Ni-Cu oxides mixed with low-temperature (black) and high-temperature (green) NiO), was associated with respiratory carcinogenicity. Conversely, exposures to approximately ≤ 1 mg Ni/m^3 of silicate oxides and complex Ni oxides (largely free of Cu) did not result in any nickel-related respiratory cancer risk.

The animal data suggest that high-temperature green NiO is a weakly positive carcinogen in rats by inhalation, with negative or equivocal evidence of carcinogenicity in mice. It is possible that the lung tumors seen in rats exposed to green NiO may have been generated by an inflammatory/proliferative response that results from the impaired function and chronic activation of macrophages, rather than by a direct heritable effect of Ni^{2+} ions. At present, it is not known if this high-concentration, low-solubility particle effect can occur in humans. Compared to nickel subsulfide, high temperature, green NiO appears to pose a lower risk for respiratory carcinogenicity. The relative carcinogenic potential of other nickel oxides (NiO) and complex oxides will likely depend on their concentration, solubility and ease of phagocytosis/endocytosis.

With regard to carcinogenicity assessment, it seems appropriate to draw a distinction between two groups of nickel oxides. An NTP category of "*known human carcinogen*" seems appropriate for Ni-Cu oxides as well as high and low temperature NiO found in nickel refineries; while silicate oxides and complex nickel oxides (devoid of copper) found in nickel using industries can best be classified as "*reasonably anticipated to be a carcinogen.*"

5.3. SOLUBLE NICKEL

The human evidence does not establish that soluble Ni compounds by themselves act as complete respiratory carcinogens. In the ICNCM Report [2], there were no cohorts where exposure was solely to soluble nickel compounds. While the Report concluded that exposures to soluble nickel compounds (predominantly in excess of 1 mg Ni/m^3) were associated with excess respiratory cancers, the authors suggested the possibility that the role of soluble nickel may be one of enhancement, since the evidence for soluble nickel compounds being carcinogenic was inconsistent across cohorts. The recent negative rodent NTP inhalation studies of nickel sulfate hexahydrate appear to confirm that soluble nickel compounds, by themselves, are not likely to cause respiratory tumors.

The relevance of the animal data for human extrapolation has been questioned on the grounds that the highest concentration to which rats were exposed was 0.1 mg Ni/m^3 while workers in some of the cohorts studied by the ICNCM experienced soluble nickel exposures ≥ 1 mg Ni/m^3. It should be noted that the aerosol used in the NTP studies (mist) had an average size of 2-3 μm while the particle size of the aerosols in the workplace has a much larger distribution with aerosols of 2-3 μm comprising less than 5%

of the total. Preliminary results from an animal to human extrapolation study based on deposition/clearance models for rat and human lungs, indicate that after accounting for particle size distribution, the exposures experienced by the rats in the NTP studies appear equivalent (in terms of nickel lung burden) to those experienced by workers in the epidemiologic nickel refinery studies [98].

In the lung, soluble nickel compounds are not endocytized; rather, they dissociate to release nickel ions and are rapidly cleared from the lungs. Ni^{2+} ions may cross the cell membrane using the Mg^{2+} ion transport system, as has been seen in microorganisms [99]. Because Mg^{2+} ions are present in cells at mM levels, high concentrations of Ni^{2+} ions are needed to compete with Mg^{2+} ions for their uptake [100]. It is possible to speculate that if the dose were sufficiently high (as happens in *in vitro* assays) enough Ni^{2+} ions could reach the nucleus to have an effect. This is unlikely *in vivo* since the toxic effects of soluble nickel compounds [46] would be evident long before a sufficiently high concentration of Ni^{2+} ions in the nucleus could be achieved.

The solubility of nickel sulfate hexahydrate in biological fluids results in release of Ni^{2+} ions at the bronchioalveolar surface, causing cell toxicity and some inflammation. Proliferation rates are enhanced, but given that the background (spontaneous) number of initiated cells is presumed to be very low, and many of these cells could be killed by the toxic effects of Ni^{2+} ions, no tumors are expected to develop. Because soluble nickel compounds may stimulate cell proliferation (the second component of the cancer process), they may act as enhancers of other compounds that are able to induce heritable changes. Furthermore, the presence of soluble nickel compounds could adversely affect the macrophage-mediated clearance of more insoluble nickel compounds [79].

With regard to carcinogenicity assessment, because soluble nickel compounds (such as hydrated nickel sulfate and nickel chloride) do not appear to be carcinogenic by themselves, they should not be listed as either "*known*" or "*reasonably anticipated to be a carcinogen.*" Because soluble compounds may affect only one of the components of the carcinogenic process (cell proliferation), they present negligible risk of carcinogenicity acting alone.

5.4. METALLIC NICKEL (ELEMENTAL NICKEL AND NICKEL ALLOYS)

Epidemiologic studies have not shown an association between the relatively low ($\leq$ 1 mg Ni/m^3) metallic nickel exposure found in industrial settings and respiratory carcinogenesis. Animal evidence regarding the potential carcinogenicity of metallic nickel by a relevant route of exposure is limited but suggests the absence of respiratory carcinogenic risk.

For metallic nickel, as for nickel subsulfide, the release of Ni^{2+} ion is not based on solubility. Rather, deposited or endocytized particles need to be oxidized to release Ni^{2+} ions. The particle size and the presence of oxidants in the lung surface and inside the cells could influence the kinetics of this reaction. Small size particles are expected to have a higher release of Ni^{2+} ion resulting in greater toxicity but faster lung and renal clearance than larger particles.

In addition, the presence of other metals in Ni-containing alloys may increase or decrease the rates of oxidation and release of Ni^{2+} ions. Therefore, more research is needed to determine the relative rates of nickel corrosion from elemental nickel and individual alloys under biologically relevant conditions.

With regard to carcinogenicity assessment, no NTP classification is justified. Past and current exposures to metallic nickel particles in occupational settings do not appear to pose a respiratory cancer risk for humans. Thus, rather than elevating it to the "*known*" category, NTP should remove metallic nickel from the list of substances that are "*reasonably anticipated to be a carcinogen.*"

5.5. NICKEL CARBONYL

Exposure to nickel carbonyl can result in severe acute respiratory damage. The extreme acute toxicity of this compound has resulted in its use in closed circuit applications that limit human contact with nickel carbonyl to accidental exposures. Therefore, there is a paucity of either human or animal data on the potential effects of chronic exposure to nickel carbonyl. Review of the limited information that is available demonstrates an absence of human evidence for the carcinogenicity of nickel carbonyl. The equivocal evidence of a carcinogenic effect of nickel carbonyl comes from animal studies where exposures clearly exceeded the Maximum Tolerated Dose (MTD). Therefore, NTP should not categorize nickel carbonyl as either a "*known human carcinogen*" or a compound that is "*reasonably anticipated to be a carcinogen.*"

6. Conclusions

Examination of the *in vitro*, animal and epidemiologic data indicates that **speciation is of paramount importance for assessing the respiratory carcinogenicity of individual nickel species**. The concentration as well as the ability of nickel compounds to be phagocytized/endocytized and their *in vivo* solubility may be the most important factors in determining the bioavailability of Ni^{2+} ions at target sites in the nucleus of respiratory tract cells, and hence, the respiratory carcinogenic potential of these compounds.

The nickel species discussed in this paper have very different biological behaviors. With regard to carcinogenicity assessment:

- Sulfidic nickel (including nickel subsulfide) could appropriately be included by NTP in the "*known human carcinogen*" category. Because nickel subsulfide may efficiently affect both components of the carcinogenic process, this compound appears to present the highest respiratory carcinogenic potential relative to other nickel compounds.

- A distinction should be drawn between two groups of nickel oxides. An NTP category of "*known human carcinogen*" seems appropriate for the mixtures of Ni-Cu oxides, high-temperature NiO, and low-temperature NiO found in nickel refineries; while silicate oxides and complex nickel oxides (devoid of copper) found in nickel using industries can best be classified as "*reasonably anticipated to be a carcinogen.*"

  When low-temperature (black) or high-temperature (green) NiO are not mixed with Ni-Cu oxides (as they were in the nickel refineries) their carcinogenic potential is less clear. High-temperature green NiO is a weakly positive carcinogen in rats by inhalation. It is possible that the lung tumors seen in rats exposed to this compound were generated as a consequence of a particle effect, rather than by a direct heritable effect of Ni^{2+} ions. At present, it is not known if this high-concentration, low-solubility particle effect can occur in humans. The carcinogenic potential of other individual nickel oxides may depend on their concentration, manufacturing history, and solubility.

- Soluble nickel compounds (such as hydrated nickel sulfate and nickel chloride) should not be listed as either "*known*" or "*reasonably anticipated to be a carcinogen.*" Because soluble nickel compounds may affect only one component of the carcinogenic process (cell proliferation), they a present negligible risk of carcinogenicity acting alone.

- For metallic nickel, no NTP classification is justified. Past and current exposures to metallic nickel particles in occupational settings have shown no respiratory cancer risk for humans.

- An NTP category of "*known human carcinogen*" for nickel carbonyl is totally unjustified. This is based on the absence of human evidence for the carcinogenicity of nickel carbonyl and the limited

animal carcinogenicity studies. These studies caused high mortality in all the exposure groups (clearly exceeding the Maximum Tolerated Dose) resulting in an equivocal carcinogenic effect.

For the reasons set forth above, *Nickel and Nickel Compounds* should not be listed as a "*known human carcinogen*" in the NTP Ninth Biennial Report on Carcinogens. Instead, NTP should make species-specific carcinogen determinations for the various forms of nickel, as suggested above.

7. References

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